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CASE REPORT

IMMUNOLOGICAL RESPONSE IN CASES OF COMPLICATED AND UNCOMPLICATED BARTONELLOSIS DURING PREGNANCY

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SUMMARY

Bartonellosis (Carrion's Disease) during pregnancy is associated with high rates of maternal and perinatal mortality. We report the immunological patterns in two cases of human bartonellosis during pregnancy. One patient had an uncomplicated course while the second patient developed life threatening anasarca and cardiac tamponade. The patient with a complicated course had a Th1 response with a higher elevation of IL-10. This elevation has been associated with poor outcome pregnancies during bacterial infections.

KEYWORDS: Carrion's Disease; Pregnancy; Immunology.

INTRODUCTION

Infection with *Bartonella* sp. has been associated with Cat Scratch Disease, Bacillary Angiomatosis, endocarditis, Bacillary Peliosis, Carrion's Disease, etc[7]. In Peru, Carrion's Disease has been described since the pre-Columbian cultures[1]. The female sandfly of the genus *Lutzomyia* spp. is the vector of transmission[12], and has been mainly reported between 500 and 3200 meters of elevation[12]. The etiologic agent of Carrion's Disease is *Bartonella bacilliformis*, an aerobic, pleomorphic and monopolar flagellated Gram-negative bacterium which is surrounded by pilli and aggregative fimbriae[11]. These bacteria may produce an acute infection (Oroya Fever) or chronic infection (Peruvian Wart) with differing clinical presentations.

The acute or hematic phase is characterized by fever and a severe hemolytic anemia with hepatosplenomegaly, jaundice and pallor. The mortality of the acute phase varies between 1% in hospitalized patients and 88% in untreated patients[12].

During the acute phase, non-understood cellular immunosuppression predisposes patients with bartonellosis to secondary infection and occurs due to transient alteration in T- lymphocyte number and function, and is not accompanied by humoral immunodeficiency[3,13].

Complications of Carrion's Disease include myocarditis, pericarditis, neurobartonellosis, and superimposed infection due to the previously mentioned immunosuppression. Co-infections with *Salmonellas*, toxoplasmosis, tuberculosis, pneumococcal pneumonia, *Pneumocystis carinii* pneumonia, and *Staphylococcus aureus* sepsis has been described[4,8]. Non-infectious complications include congestive heart failure, thrombocytopenia, severe anemia, seizures, intracranial hypertension, and multi-organ dysfunction[4,8,12].

Neonatal and maternal mortality rates are high when the acute phase of Carrion's Disease occurs during pregnancy[8,18]. Trans-placental infection has also been reported[8]. In one small case series, two of five pregnant women with the acute phase of Bartonellosis died and two of the remaining patients had a spontaneous abortion[8].

The eruptive phase of Bartonellosis is characterized by the development of either a single wart or hundreds of warts that may resemble Bacillary Angiomatosis or Kaposi's Sarcoma[7,8,12]. Among three pregnant women with the eruptive phase, none of these patients or their newborns had complications[7].

CASES REPORTS

CASE 1: 27 year-old G2 P1 Hispanic female farmer, from Huaraz (Ancash-Peru), at 16 weeks estimated gestational age by last menstrual period presented with a one-month history of nausea, vomiting, malaise, weakness, fever, jaundice and 12 kilogram weight loss. In Huaraz the peripheral smear showed that 5% of red blood cells were infected. She was initially treated with Ceftriaxone, Penicillin and Azithromycin during seven days. Due to a partial response, the antibiotic treatment

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Table 1
Immunological pattern in two cases of Bartonellosis during pregnancy at the acute phase (AP) and post resolution of the infection (PI)

Patient	Moment of study	WBC (cel/μL)	CD4+ (cel/μL)	CD8+ (cel/μL)	CD4/CD8	IFN-γ (pg/mL)	TNF-α (pg/mL)	IL-4 (pg/mL)	IL-10 (pg/mL)
Case 1	AP	31800	2873.93	1643.26	1.75	533.75	1.09	0.00	69.64
	PI	12050	492	745	0.66	188.78	0.00	0.00	50.85
Case 2	AP	6200	780	310	2.57	18.75	0.91	2.33	12.07
	PI	7850	1190	506	2.35	20.63	2.99	11.47	18.90

was modified to Chloramphenicol and Clindamycin during four days. This patient subsequently developed diarrhea and hypotension requiring transfer to a tertiary care hospital in Lima. On arrival the patient was febrile, hypotensive, hypoxic, and had anasarca, with 40% of red blood cells infected by *B. bacilliformis*. An echocardiogram revealed a cardiac tamponade. Obstetric ultrasound showed a gestation of 18 weeks six days, AFI = 31mm, FHT = 157/min. Patient had a pericardiotomy the next day and 500 cc of hematic pericardic fluid was drained during 24 hours. The developed fever, hypotension and respiratory distress two days later. The patient received Chloramphenicol, Clindamycin and Ceftaxidime with improvement of clinical condition. The patient was discharged after 15 days of hospitalization with resolution of her acute phase Bartonellosis with no evidence of complications in the fetus. She returned to Ancash, where no further follow-up was recorded. The immunology markers evaluated during the admission showed a WBC count of 31800 cel/μL, CD4+ recount of 2873 cel/μL, a CD4+/CD8+ ratio of 1.75, with very elevated values of INF-gamma (533.75 pg/mL) and IL-10 (69.64 pg/mL). Those values decreased after treatment including the CD4+, CD8+ recount, with a CD4+/CD8+ ratio of 0.66, also decreased but still elevated values of INF-gamma (188.78 pg/mL) and IL-10 (50.85 pg/mL) were found (Table 1).

CASE 2: 23 year-old G2P1 Hispanic female from Caraz (Ancash-Peru), G2 P1-0-0-1, at 13 weeks estimated gestational age by LMP, presented with a three month history of malaise, headache, emesis, osteo-mio-arthicular mild pain, diaphoresis and fever. The patient had a blood smear obtained in the emergency room, which demonstrated *Bartonella bacilliformis* in 1% of red blood cells. She received intravenous Ceftriaxone for seven days, without reduction in the proportion of infected red blood cells. Patient received an additional six days of treatment with subsequent eradication of *B. bacilliformis* in the red blood cell smear. She was discharged with resolution of her uncomplicated, acute phase of Human Bartonellosis. She subsequently delivered of 3150 grams infant with Apgar scores of 5 and 9 at 39 weeks. The immunology markers evaluated during the acute phase showed normal values for WBC count (6200 cel/μL), CD4+ recount (780 cel/μL), CD4+/CD8+ ratio (2.57), INF-γ (18.75 pg/mL) and IL-10 (12.07 pg/mL). After treatment, a mild increase of the WBC, CD4+, and CD8+, with a CD4+/CD8+ ratio of 2.35 was found. Also only a mild increase in the anti-inflammatory cytokines was noted (Table 1).

METHOD

Samples were collected in two local hospitals in the department of Huaraz, Peru. Cases were confirmed according guidelines of the Peruvian Ministry of Health, using peripheral smear and blood culture, in both cases. Each specimen was collected in a 10 mL vacutainer tube with EDTA®, and send immediately to Lima to their analysis. All the samples arrived to the laboratory in less than 12 hours.

The immunological studies were done in the Instituto de Medicina Tropical "Alexander von Humboldt" (IMT-AvH), of the Universidad Peruana Cayetano Heredia. The absolut recount of T cells $CD4^+$/ T cells $CD8^+$ were performed in total peripheric blood using Flow-Count (Coulter®). The samples were analyzed using the program CELLQuest in a Cytometer FACSCalibur (Becton Dickinson®). The production of IFN-γ, TNF-α, IL-4, and IL-10, were evaluated in the plasma of each patient. Commercial Kits were used (BD OptEIA™ ELISA Kits®). The concentration of cytokines (pg/mL) was measured using an "Elisa Reader" (Biorad®).

DISCUSSION

We have presented two cases of acute phase of Carrion's Disease during pregnancy and for the first time their immunology response pattern during the acute phase at the moment of their admission and a second pattern during their recovery before to be discharged from the hospital. It is well known that during pregnancy, the evolution of the acute phase of Human Bartonellosis has a high incidence of adverse poor maternal and fetal outcomes. There is a high incidence of miscarriage after the acute infection of *Bartonella bacilliformis*[4,7,8,18], although the etiology is unclear.

Pregnancy may potentially alter the regulation of the inflammatory response to Bartonellosis. Immunoglobulin synthesis in pregnant women is increased, whereas cell-mediated response is decreased, which may allow increase susceptibility to intracellular pathogens during pregnancy[16]. During pregnancy, progesterone-induced blocking factor (PIBF) is released and mediates the immunomodulatory and anti-abortive effect of progesterone due to the production of Th2 type cytokines[2,5]. High levels of IL-4, IL-6, IL-10 and PIBF have been founded in health pregnant women, and increased IL-2, INF-gamma, and low expression on PIBF and IL-10, in those pregnancies with high risk for prematurity or recurrent spontaneous miscarriage[14,15,17].

In the first case, the patient with complicated course of Carrion's Disease, showed an increase of pro-inflammatory cytokines. During pregnancy Th1 cytokines may be harmful, as TNF-α has been demonstrated to inhibit trophoblast cell proliferation. In patients with severe sepsis, the measure of IL-1 and TNF-α correlates negatively with the survival rate of the patient[2,15,16]. Those cytokines are expressed mainly in the Th1 response. The pro-inflammatory response may be a

cause of miscarriage during severe infections of *Bartonella bacilliformis*, as was observed in other gram negative infections[2,5].

The second patient with uncomplicated acute phase of Carrion's Disease did not demonstrate an increase in Th1 cytokines but did have an increased level of the Th2 cytokine IL-4.

Both patients showed and elevation of IL-10, but it was most evident in the patient with the complicated course. Elevation of IL-10 is due mainly by T cells CD43+ CD4+, which are important cells in the equilibrium of the Th1 or Th2 response[6,14]. Th2 cytokines, including IL-10, inhibit the secretion of Th1 cytokines[16], and as previously mentioned may have "anti-abortive effect" in a normal pregnancy. However, high levels of IL-10 had been reported in patients with severe sepsis, mainly by gram-negative organism[9,10], as a response to the effect of inflammatory cytokines, and this may produce an "immunological paralysis" of antigen presenting cells, and macrophages. This phenomenon may explain the more severe course of Human Bartonellosis in some patients, and deserves further study.

CD4+ T cell recount were within normal range in both patients. This may indicate that the hypothesized decrease in cell-mediated immunity response in pregnant women with *Bartonella bacilliformis* infection is due to a dysfunction in the cell mediated response rather than a loss of the number of T cells involved in this response.

Our results showed that the patient that developed the complicated course had mainly a Th1 response, with elevation of pro-inflammatory cytokines that is associated with poor outcome pregnancies. The patient who developed a non-complicated course, showed a mild elevation of pro inflammatory and elevated anti-inflammatory cytokines. In both cases, IL-10, an anti-inflammatory cytokine, was elevated but more prominently in the complicated patient, and the possibility that an "immunological paralysis" phenomenon occurred in Human Bartonellosis deserves further investigation.

RESUMEN

Respuesta inmunologica en casos de bartonelosis con y sin complicaciones durante el embarazo

Bartonelosis (Enfermedad de Carrión) durante el embarazo esta asociado a una alta tasa de mortalidad maternal y perinatal. Reportamos el perfil inmunológico de dos casos de Bartonelosis humana en el embarazo. Una paciente tuvo un curso sin complicaciones, mientras la segunda presento complicaciones severas de anasarca y tamponamiento cardiaco. La paciente con curso complicado tuvo un patrón de repuesta Th1, con una elevación de IL-10, que se ha asociado a mal pronóstico en infecciones durante embarazo.

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